Alcohol Production by Selected Yeast Strains in Lactase-Hydrolyzed Acid Whey

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Summary

Ethanol production by *Kluyveromyces fragilis* and *Saccharomyces cerevisiae* was studied using cottage cheese whey in which 80 to 90% of the lactose present had been prehydrolyzed to glucose and galactose. Complete fermentation of the sugar by *K. fragilis* required 120 hr at 30°C in lactase-hydrolyzed whey compared to 72 hr in nonhydrolyzed whey. This effect was due to a diauxic fermentation pattern in lactase-hydrolyzed whey with glucose being fermented before galactose. Ethanol yields of about 2% were obtained in both types of whey when *K. fragilis* was the organism used for fermentation. *Saccharomyces cerevisiae* produced alcohol from glucose more rapidly than *K. fragilis*, but galactose was fermented only when *S. cerevisiae* was pregrown on galactose. Slightly lower alcohol yields were obtained with *S. cerevisiae*, owing to the presence of some lactose in the whey which was not fermented by this organism. Although prehydrolysis of lactose in whey and whey fractions is advantageous in that microbial species unable to ferment lactose may be utilized, diauxie and galactose utilization problems must be considered.

INTRODUCTION

The disposal of whey, a by-product of cheese manufacture, has become a serious pollution problem in some areas. In 1974, 32.5 billion pounds of whey were produced, over half of which was disposed of as waste.[1] This represents a pool of 1.6 billion pounds of lactose, which if converted into usable food products, could be of sizable monetary value to the dairy industry. Since lactose is the major component of whey solids,[2] it must be taken into consideration in any effective utilization process.

Utilization of whey as a fermentation substrate has been studied extensively. The most promising processes utilize whey as a sub-

* Agricultural Research Service, U.S. Department of Agriculture.

strate for the production of yeast,[3,4] alcohol,[5] and alcoholic beverages.[6,7]

A major problem in using whey as a fermentation medium has been the fact that relatively few organisms are able to ferment lactose. Early research,[5,8,9] in which a number of lactose fermenting yeasts were screened for their fermentation efficiency in whey containing 5% lactose, indicated *Torula cremoris* to be the most efficient under the conditions studied. This finding was not substantiated by Yoo,[10] who evaluated the relative efficiency of lactose conversion to alcohol by five yeast cultures, including *T. cremoris*. Yoo found that in acid whey containing 10% total solids, *Saccharomyces fragilis* was the most efficient lactose fermenter. However, only 55% of the available lactose was converted, possibly owing to an inability of the yeast to tolerate alcohol. If the lactose in whey could be hydrolyzed into its constituent monosaccharides glucose and galactose, non-lactose fermenting organisms such as strains of *S. cerevisiae* that tolerate high concentrations of alcohol could be used for alcohol fermentations.

Thompson and co-workers[11,12] have demonstrated that there may be economic advantages in manufacturing cheddar and cottage cheese from milk which has been treated with β-galactosidase to hydrolyze the lactose present. The whey obtained from these processes contains at least 70% hydrolyzed lactose. Our study was initiated to evaluate the potential for ethanol production from cottage cheese whey derived from β-galactosidase treated milk. We utilized *S. cerevisiae*, an organism which is employed in the manufacture of wine and beer. On the basis of Yoo's work,[10] *Kluyveromyces fragilis* was selected for comparison.

MATERIALS AND METHODS

Organisms

Kluyveromyces fragilis NRRL Y-1109 (formerly classified as *Saccharomyces fragilis*) and *S. cerevisiae* ATCC 834 were the organisms used in this study. Stock cultures were maintained on YM agar (Difco)* slants at 4°C. Active cultures for inoculation were prepared by growing the test organism in YM broth in still culture at 25°C for 72 hr and then transferring to fresh YM broth. This culture was then grown for an additional 48 hr with shaking (200 rpm) at 25°C. Adaptation of the yeast strains to either glucose, galactose, or

* Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

lactose was accomplished by growing the organisms in YM broth containing the sugar desired prior to inoculation into the whey medium.

Whey

Cottage cheese whey, containing over 80% of its lactose in the hydrolyzed form, was prepared as follows: Freshly separated skim milk, pasteurized at 61°C for 30 min, was cooled to 31°C and treated with 0.3 g/liter Maxilact food grade lactase enzyme (β-galactosidase) (Enzyme Development Corp., New York, N.Y.). The milk was then immediately inoculated at a level of 5 to 6% with an active lactic starter culture consisting of *Streptococcus lactis* and *Streptococcus cremoris* (Marschall Division, Miles Laboratories Inc.). After starter addition, 2.5 μl/liter Marzyme double strength coagulant (Marschall Division) was added to produce a firmer curd. When the pH of the coagulum reached 4.6, the curd was cut and cooked at 49–51°C for 10 min and then cooled. The whey was drained from the vat, pasteurized at 61°C for 30 min, and stored frozen until needed. Cottage cheese whey, drained from cottage cheese prepared from skim milk without lactase treatment, was obtained for a control.

Fermentation Apparatus

Fermentation was carried out in sterile Nephlo flasks equipped with rubber septums over the sidearms and fitted with rubber stoppers containing L-shaped pieces of glass tubing plugged with cotton wool at one end. This arrangement permitted the Nephlo flasks to be connected to trapping flasks, whose exit ports were submerged in water by means of Tygon tubing in order to allow carbon dioxide formed during fermentation to escape while maintaining anaerobic conditions.

Cultural Conditions for Ethanol Production

Control and lactase-hydrolyzed cottage cheese wheys were thawed, and 200 ml aliquots were placed in Nephlo flasks and repasteurized at 83°C for 5 min. After cooling, the whey media were inoculated at a level of 1% with a 48 hr culture of either *K. fragilis* or *S. cerevisiae* and incubated in a thermostatically controlled waterbath set at 30°C. At intervals during the fermentation period, samples for microbial and chemical analysis were aseptically withdrawn from the flasks through the septums using sterile syringes. The samples for chemical analysis were centrifuged at 22,000 *g* in a Sorvall Superspeed RC-2 Centrifuge to remove yeast cells and precipitated protein.

ANALYTICAL PROCEDURES

Growth Studies

Enumeration of cell populations during growth and fermentation was carried out by pour plate counts on YM agar (Difco). Serial dilutions were made in 0.1% peptone water. Plates were incubated at 30°C and counted after 48 hr.

pH

pH measurements were made using a Radiometer pH meter, Model 22 (Radiometer, Copenhagen) equipped with a Calomel combination electrode.

Alcohol Analyses

Alcohol was determined by the alcohol dehydrogenase procedure described in the Boehringer-Mannheim Corp. catalog.[13] Alcohol dehydrogenase (Sigma Corp.) was used at a concentration of 10 mg/ml. Assay solutions were incubated at 25°C for 90 min and then read at 340 mμ using a Zeiss Model PMQ II spectrophotometer. Appropriate dilutions of absolute alcohol were used to prepare a standard curve.

Total Reducing Sugars

Total reducing sugar was determined by the colorimetric Folin-Wu procedure as modified by Bausch and Lomb.[14] A standard curve prepared from dilutions of lactose was used to calculate the reducing sugar content of fermentation media prepared with control cottage cheese whey. Glucose was used as a standard for lactase-hydrolyzed whey. All readings were made at 430 mμ, using a Bausch and Lomb Spectronic 20 colorimeter.

Glucose

Glucose was determined using the Salomon and Johnson reagent as described by Jasewicz and Wasserman[15] with the following modifications: to 0.1 ml of sample containing 15–150 μg of glucose were added 2.0 ml water and 1.5 ml Salomon and Johnson reagent. The color was allowed to develop at room temperature for 1 hr. Optical density was read at 635 mμ using a Zeiss Model PMQ II spectrophotometer. A standard curve was prepared using known dilutions of glucose for calculation of results.

Galactose

Galactose was determined by an enzymatic method utilizing galactose dehydrogenase as described in the Boehringer-Mannheim Corp. catalog.[13] The procedure was modified as follows: The assay medium consisted of 0.84 ml 0.1*M* Tris-HCl buffer, pH 8.6; 0.05 ml β-NAD, 5 mg/ml (Sigma Corp.); 0.10 ml sample, and 0.01 ml β-galactose dehydrogenase (Sigma Corp.) diluted to approximately 3 U/ml. The assay solutions were incubated at 25°C and read at 340 mμ using a Zeiss Model PMQ II spectrophotometer. Calculations were made from a previously prepared standard curve using galactose concentrations of 50–250 μg/ml.

Total Monosaccharides

Total monosaccharide concentration was determined colorimetrically using the procedure of Tauber and Kleiner.[16] An equimolor mixture of glucose and galactose served as a standard. Optical density was read at 420 mμ using a Bausch and Lomb Spectronic 20 colorimeter.

Lactic Acid

Lactic acid was determined using the colorimetric procedure of Lawrence,[17] which measures total lactate concentration. Appropriate dilutions of a stock solution containing lithium lactate solution were used to prepare a standard curve. Optical density was read at 505 mμ with a Bausch and Lomb Spectronic 20 colorimeter. Analysis of standards containing increasing amounts of ethanol indicated no interference with color development under the conditions used.

Data Analysis

The adjustment phase duration data were subjected to linear regression analysis and analysis of variance. Exponential growth rates and maximum population size were analyzed by linear regression and the *T* test. Alcohol production rates were analyzed by linear regression and the *T* test. Residual sugars in the fermentation medium and total alcohol yields were analyzed by the *T* test. Lactic acid concentrations in the whey were analyzed by the *T* test.

RESULTS

Growth of *K. fragilis* in whey was enhanced when the lactose was prehydrolyzed to glucose and galactose with β-galactosidase. Growth characteristics of glucose-pregrown *K. fragilis* in lactase-hydrolyzed whey as compared with normal whey are presented in Figure 1.

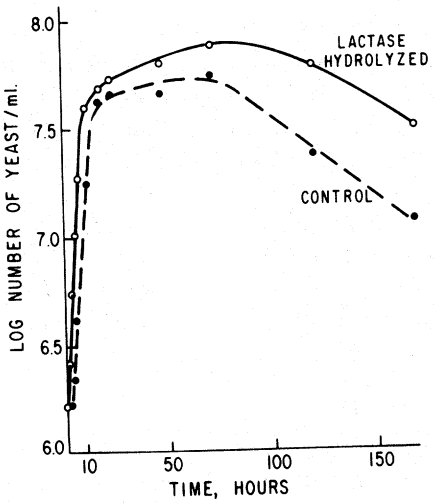

Fig. 1. Growth of *K. fragilis* in control and lactase-hydrolyzed acid wheys.

The lag phase of growth (defined in this study as the time required for the initial cell population to double) was significantly shorter ($\alpha = 0.005$) in lactase-hydrolyzed whey. The mean lag time was 2.83 hr, compared to 5.55 hr in the control. In addition, the maximum population attained was higher in the lactase-hydrolyzed whey.

Growth of *K. fragilis* in lactose-containing YM broth prior to transfer into control whey resulted in a decrease in the lag phase of growth from 5.55 to 3.68 hr. Exponential growth rates and maximum cell populations were not affected, however.

Ethanol Production by K. fragilis in Lactase-Hydrolyzed Whey

In order to determine the effect of prehydrolysis of lactose in whey on the rate of ethanol production, the control and lactase-hydrolyzed wheys were inoculated with glucose- or lactose-pregrown *K. fragilis* and fermentation was allowed to proceed. Curves illustrating alcohol production by glucose-pregrown *K. fragilis* in control and lactase-hydrolyzed wheys are presented in Figure 2. Ethanol production was more rapid in lactase-hydrolyzed whey during the first 24 hr of fermentation (significant at $\alpha = 0.001$). After 24 hr of fermentation, however, alcohol production in lactase-hydrolyzed whey decreased so that the rate of production was less than that observed in the control whey. Consequently, maximum ethanol production was attained in 72 hr in the control whey, compared to 120 hr in the lactase-hydrolyzed whey. Pregrowth of *K. fragilis* on lactose had no

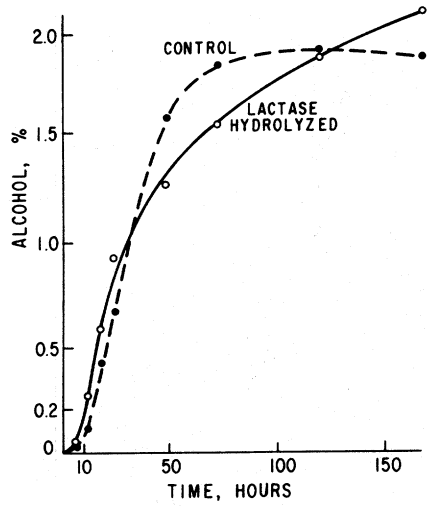

Fig. 2. Alcohol production by glucose-pregrown *K. fragilis* in control and lactase-hydrolyzed acid wheys.

effect on alcohol production rates in lactase-hydrolyzed whey. Alcohol production in control whey, however, was somewhat accelerated when *K. fragilis* was pregrown on lactose. Total yield of alcohol was similar in all cases and averaged 1.92%.

Sugar Utilization by K. fragilis during Fermentation of Lactase-Hydrolyzed Whey

In order to characterize total sugar utilization during fermentation of control and lactase-hydrolyzed wheys by *K. fragilis*, the disappearance of total reducing sugars during the course of the fermentation was monitored. The results for glucose-pregrown *K. fragilis* are shown in Figure 3. Reducing sugars decreased at about the same rate (no statistically significant difference even at $\alpha = 0.5$) in both the control and lactase-hydrolyzed wheys during the first 24 hr of fermentation. In the later stages, however, the rate of sugar utilization in the lactase-hydrolyzed whey decreased to a level less than that of the control, so that fermentation was completed 48 hr earlier in the control whey.

The pattern of utilization of the individual sugars in lactase-hydrolyzed whey was determined by monitoring changes in glucose, galactose, and total monosaccharide concentrations during the fermentation period. The results of these analyses are illustrated in Figure 4. Examination of the curve representing the decline in the

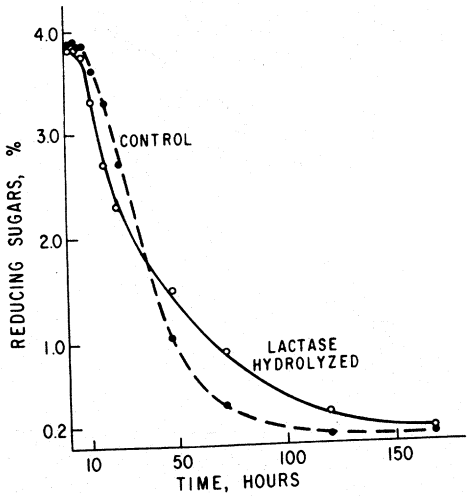

Fig. 3. Change in concentration of total reducing sugars during fermentation of control and lactase-hydrolyzed acid wheys by glucose-pregrown *K. fragilis*.

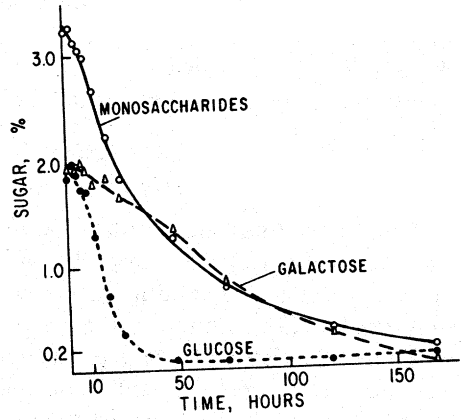

Fig. 4. Change in total monosaccharide, glucose, and galactose concentrations during fermentation of lactase-hydrolyzed acid whey by *K. fragilis*.

concentration of total monosaccharides over time shows that a sharp decrease occurred during the first 24 hr of fermentation followed by a more gradual decline during the remainder of the fermentation period. Analyses for glucose and galactose, individually, showed that although glucose disappeared rapidly and completely during the first 24 hr of the fermentation, there was little change in galactose concentration during this interval. Galactose began to disappear

from the fermentation medium during the second 24 hr of the fermentation and had almost completely disappeared after 120 hr had elapsed. These results clearly demonstrate that a diauxic pattern of sugar fermentation took place in the lactase-hydrolyzed whey with virtually complete fermentation of glucose occurring before fermentation of galactose was initiated.

Growth of S. cerevisiae in Lactase-Hydrolyzed Acid Whey

Strains of *S. cerevisiae,* a nonlactose fermenting yeast, are commonly used in commercial beer and wine-making processes because of their ability to withstand relatively high levels of alcohol. Because of this alcohol tolerance, we decided to investigate the potential of *S. cerevisiae* for use in an alcohol fermentation of lactase-hydrolyzed acid whey.

Growth curves of glucose- and galactose-pregrown *S. cerevisiae* in lactase-hydrolyzed acid whey are presented in Figure 5. Duration of the lag phase of growth for glucose-grown *S. cerevisiae* in lactase-hydrolyzed acid whey was 5.76 hr, which was significantly longer ($\alpha = 0.005$) than that for *K. fragilis* under the same conditions (2.73 hr). In addition, the maximum population attained by *S. cerevisiae* in lactase-hydrolyzed whey was significantly lower than that for *K. fragilis.* Cell numbers of *S. cerevisiae* reached a maximum at 24 hr and declined sharply thereafter. As shown by the curve in

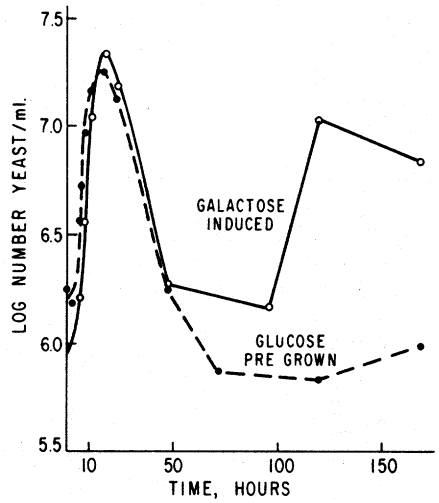

Fig. 5. Growth of glucose- and galactose-pregrown *S. cerevisiae* in lactase-hydrolyzed acid whey.

Figure 5, when the organism was pregrown on galactose, however, cell numbers increased again between 72–120 hr after the initial decline at 24 hr.

Alcohol production by glucose- and galactose-pregrown *S. cerevisiae* in lactase-hydrolyzed whey is shown in Figure 6. When *S. cerevisiae* was pregrown on glucose, alcohol production in lactase-hydrolyzed acid whey ceased after 24 hr. The maximum ethanol yield attained with glucose-pregrown cells of *S. cerevisiae* was 0.85%, an amount less than half of that obtained with *K. fragilis* under similar conditions. After 48 hr of incubation, alcohol levels began to decline and continued to do so throughout the remainder of the incubation period. When the organism was pregrown on galactose, however, alcohol production ceased at 24 hr but was then reinitiated between 72 and 120 hr of incubation reaching a maximum at 120 hr. However, the maximum alcohol yield obtained was only 1.65%, which although twice the yield obtained with glucose-pregrown *S. cerevisiae*, is still significantly ($\alpha = 0.001$) below the 2% yield obtained with glucose-pregrown *K. fragilis* under similar conditions.

Patterns of sugar utilization during fermentation of lactase-hydrolyzed whey by glucose- and galactose-pregrown *S. cerevisiae* were analyzed in order to define their relation to alcohol production. Changes in the concentrations of total reducing sugars, monosaccharides, glucose, and galactose during fermentation of lactase-hydrolyzed whey when *S. cerevisiae* was pregrown on glucose are shown in Figure 7.

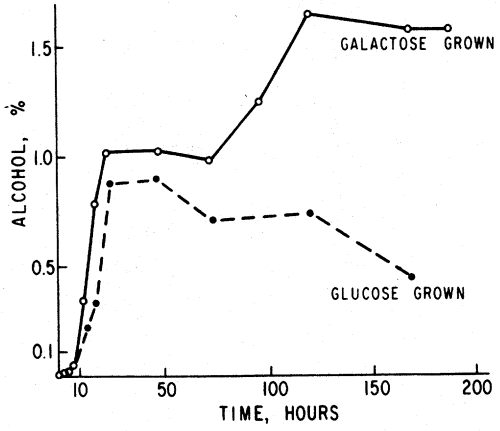

Fig. 6. Alcohol production by glucose- and galactose-pregrown *S. cerevisiae in* lactase-hydrolyzed acid whey.

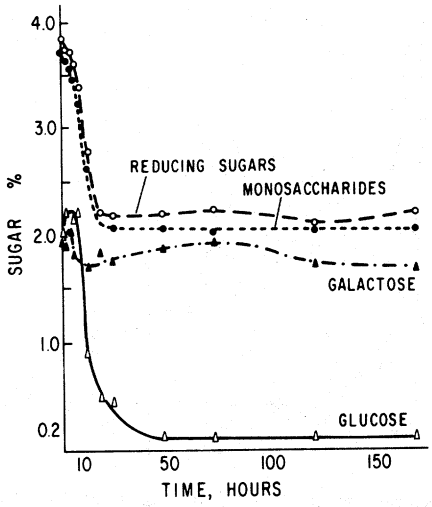

Fig. 7. Change in concentration of total reducing sugar, total monosaccharides, glucose, and galactose during fermentation of lactase-hydrolyzed acid whey by glucose-pregrown *S. cerevisiae*.

The reducing sugar and monosaccharide concentrations declined by approximately half during the first 24 hr of the incubation and then underwent no further change. Measurement of the individual monosaccharides showed that although glucose disappeared almost completely within 24 hr, galactose levels did not change during the entire fermentation period. These results demonstrate that glucose-pregrown *S. cerevisiae* was unable to utilize galactose even after 168 hr of incubation. Therefore, the low ethanol yields obtained in lactase-hydrolyzed whey fermented by glucose-pregrown *S. cerevisiae* are the maximum to be expected under these conditions.

Sugar utilization patterns for galactose-pregrown *S. cerevisiae* are shown in Figure 8. Total reducing sugars and monosaccharide concentrations decreased rapidly during the first 24 hr and then underwent little further change until 96 hr of incubation. Glucose disappeared rapidly and completely during the first 24 hr of fermentation. Galactose, however, did not begin to disappear until after about 96 hr. These results demonstrate that *S. cerevisiae* also exhibits a diauxic fermentation pattern in lactase-hydrolyzed whey and that, furthermore, pregrowth on galactose is essential to stimulate galactose utilization by this organism in lactase-hydrolyzed whey.

Although residual glucose and galactose levels were identical in the galactose-pregrown *S. cerevisiae* and *K. fragilis* spent lactase-hydro-

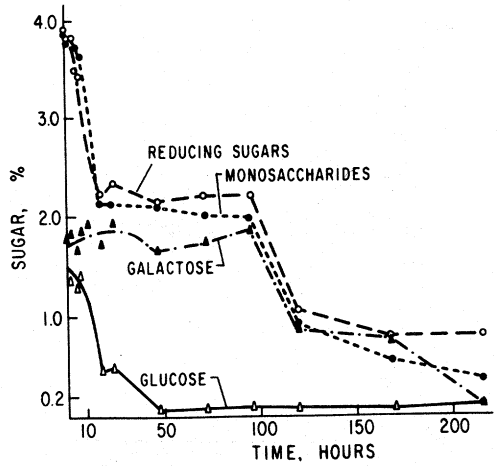

Fig. 8. Change in concentration of total reducing sugar, total monosaccharides, glucose, and galactose during fermentation of lactase-hydrolyzed acid whey by galactose-pregrown *S. cerevisiae*.

lyzed whey fermentation media, the concentration of residual reducing sugars was 0.80% in the *S. cerevisiae* fermentation broth as compared to 0.12% in the *K. fragilis* fermentation broth. This difference in residual reducing sugars (statistically significant at $\alpha = 0.001$) indicates that *K. fragilis* was able to utilize more of the total available carbohydrate than was *S. cerevisiae*.

Acidic Side-Products in Fermentation of Lactase-Hydrolyzed Whey

Lactic acid concentrations and pH of the control and lactase-hydrolyzed wheys fermented by either *K. fragilis* or *S. cerevisiae* are listed in Table I. Only a very slight decrease in pH occurred during the fermentation period under all conditions studied, and there was essentially no change in lactic acid concentrations, although the lactic acid content of lactase-hydrolyzed whey was significantly higher ($\alpha = 0.05$) than that of control whey.

DISCUSSION

Our studies with *K. fragilis* have shown that alcohol production proceeded more rapidly in lactase-hydrolyzed whey than in control whey during the period when glucose was being fermented. When the substrate was galactose, however, alcohol was produced more slowly than in the control whey. The diauxic pattern of fermentation thus resulting in lactase-hydrolyzed whey led to lengthened

TABLE I

Change in pH and Lactic Acid Concentration[a]

	pH		
		K. fragilis	*S. cerevisiae*
	Control	Lactase hydrolyzed	Lactase hydrolyzed
Initial	4.67	4.63	4.64
Final	4.51	4.47	4.45
		Lactic acid (%)	
Initial	0.57	0.71	0.63
Final	0.60	0.67	0.66

[a] This occurred during the fermentation of control and lactase-hydrolyzed acid wheys by *K. fragilis* and *S. cerevisiae*.

fermentation times over the control whey. Diauxic patterns of sugar utilization by microorganisms have been reported by other researchers.[18,19] These reports have indicated that glucose is usually preferentially utilized when a mixture of carbon sources is available. This effect has been related by some researchers to the ability of glucose to interfere with the transport of other sugars into the cell.[20,21] Rogosa[22] reported that a number of yeasts, including *K. fragilis*, fermented equimolar mixtures of glucose and galactose more slowly than lactose; he concluded that this was because galactose is normally fermented with relative difficulty.

Our studies have also shown that alcohol production in control whey can be accelerated if *K. fragilis* is pregrown on lactose prior to inoculation into the whey medium. This effect can be explained by the work of Davies,[23] who showed that lactose utilization in *S. fragilis* is an induced system brought about by growth on either lactose or galactose. Thus, our finding that the rate of ethanol production in control whey was increased when *K. fragilis* was pregrown on lactose appears to be due to induction of the lactose utilizing system in the cells during the adaptation period, thus resulting in a shorter lag period for growth and fermentation after inoculation into the whey medium.

Rogosa[22] reported that lactose is fermented at the same rate as glucose by *K. fragilis*, while Myrbach and Vasseur[24] reported that *K. fragilis* ferments lactose more rapidly than glucose. In contrast to these reports, our results showed that alcohol is produced more rapidly when glucose is the substrate than when lactose is the sub-

strate, even when the organism has been adapted to lactose. Thus, our results are in agreement with those of Yoo,[25] who reported that *K. fragilis* in model systems produces more alcohol at a faster rate from glucose than from lactose. Our results may reflect a more rapid metabolic process for the conversion of sugar to alcohol when the substrate is glucose rather than the disaccharide lactose, since the rate at which alcohol was produced was more rapid during the fermentation of glucose than of lactose, although there was no difference in the rate of disappearance of the carbohydrate source.

When *S. cerevisiae* was substituted for *K. fragilis* in the alcohol fermentation of lactase-hydrolyzed whey, not only was maximum ethanol production lower, but during the later stages of the fermentation, alcohol levels actually decreased. Yoo[25] reported that glucose adapted cultures of certain strains of *Candida pseudotropicalis* and *S. fragilis* were unable to produce ethanol from galactose and, in fact, appeared to consume alcohol which was present in the medium. Wilharm and Sach[26] also reported that overfermentation can result in a reduction in alcohol content. In view of these reports, the decline in alcohol levels we observed with glucose-pregrown *S. cerevisiae* upon prolonged incubation may be due to metabolism of the alcohol previously formed by the yeast.

Although our results show that pregrowing *S. cerevisiae* on galactose doubled the ethanol yield (from 0.85 to 1.65%) when this yeast was used for fermentation of lactase-hydrolyzed whey, still higher yields (2% maximum) were obtained by using glucose- or lactose-pregrown *K. fragilis*. Fermentation of residual lactose present in the lactase-hydrolyzed whey (present as a result of incomplete lactose hydrolysis) would appear to account for the increase in ethanol yield achieved with *K. fragilis*, since there was significantly less residual reducing sugar in the *K. fragilis* spent fermentation medium, which could not be accounted for by either glucose or galactose.

A previous report[25] had indicated that although glucose-pregrown *S. fragilis* was able to ferment lactose, it could not utilize galactose. Another report[22] showed in somewhat unusual results that lactose adapted *Torulopsis kefir* was simultaneously adapted to galactose; as a result, this yeast could ferment galactose more readily than galactose adapted strains. Our studies showed that glucose-pregrown *S. cerevisiae* was unable to utilize the galactose present in lactase-hydrolyzed whey for the production of alcohol, although glucose-pregrown *K. fragilis* was able to do so. We believe that the presence of residual lactose in the lactase-hydrolyzed whey may

have induced the galactose utilizing system in glucose-pregrown *K. fragilis*, thus enabling the organism to ferment galactose although not adapted to galactose. *Saccharomyces cerevisiae*, which is impermeable to lactose,[27] could not be induced for galactose in this manner, nor could it ferment residual lactose in the medium. Even when *S. cerevisiae* was adapted to galactose during a pregrowth period, our results indicate that incubation for an additional 72 hr after the glucose had disappeared was required before galactose utilization was initiated in lactase-hydrolyzed whey. These data suggest that the presence of, and preference for, glucose prevented galactose utilization and this effect remained in evidence for some time, even after the glucose was no longer present. Possibly some component of the galactose utilizing system had been degraded during the period of glucose fermentation and resynthesis of an essential factor or factors was necessary before galactose utilization could proceed. Therefore, fortification of the whey medium with amino acids or certain growth factors might aid in accelerating galactose utilization by galactose adapted *S. cerevisiae*.

Lactase-hydrolyzed whey contained higher concentrations of lactic acid than did control whey. O'Leary and Woychik[28] have reported finding more lactic acid in yogurts prepared from lactase-hydrolyzed milk, leading them to suggest that lactic acid levels may be elevated in lactase-treated products. Slightly higher lactic acid levels in lactase-hydrolyzed wheys may conceivably be a problem in some processes but was not a factor in our studies.

There was little change in the pH of control and lactase-hydrolyzed wheys during fermentation by the yeast strains in our study. Myers and Weisberg[29] had previously reported that *K. fragilis* rapidly converts the lactose in whey to carbon dioxide and ethanol with almost no acid or other end-products. Our results show that the alcohol fermentation of whey containing a mixture of glucose and galactose also proceeds without the formation of significant amounts of acid products when *K. fragilis* or *S. cerevisiae* are the organisms used, and thus pH control during fermentation is unnecessary.

CONCLUSIONS

Reports in the literature[30,31] have appeared suggesting that since glucose and galactose are more universally fermentable sugars than is lactose, β-galactosidase treated whey will make a better substrate for industrial fermentations. However, our investigation of the potential use of lactase-hydrolyzed whey as a fermentation medium

for alcohol production has shown that a diauxic pattern of fermentation occurred in lactase-hydrolyzed whey with the result that fermentation time was increased over that of conventional whey.

Even though diauxie constitutes a problem in the fermentative utilization of lactase-hydrolyzed whey, this material does have certain properties which would seem to be of some benefit for its use as a substrate for alcohol fermentation. Lactase-hydrolyzed whey readily lends itself to the preparation of concentrates of relatively high solids content because of the greater solubility of glucose and galactose. This attribute may result in the achievement of higher alcohol yields than is usually possible in normal whey since high alcohol-producing yeast strains which are unable to ferment lactose can be used for fermentation. Such possibilities should be further investigated.

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